IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the Application of	Examiner: M. Woodward
ROGER P. EKINS	Group Art Unit: 1815
Serial No. 08/447,820	
Filed: May 23, 1995	
For: DETERMINATION OF AMBIENT CONCENTRATIONS OF SEVERAL ANALYTES	

DECLARATION OF JOHANN BERGER

Commissioner of Patents and Trademarks
Washington, D.C. 20231

Sir:

I, Dr Johann Berger, declare as follows:

1. I am a senior director of Advanced Systems Technologies at Boerhringer Mannheim GmbH, Tutzing, Germany, responsible inter alia for investigating and acquiring the rights to ideas for my company to develop. I have worked in the diagnostics field for 16 years. I have a good command of English. My curriculum vitae accompanies this declaration.

2. I am familiar with the content of the above patent application and the work of the inventor, Professor Roger Ekins in the field of immunoassay and, in particular, his "ambient analyte" methodology. I am also familiar with the objections raised by the Examiner in the Office Action of September 2, 1997. I have been asked to comment on the Examiner's assertion that the above application would have been obvious to a person of ordinary skill in the art in view of Professor Ekins' earlier application, WO 84/01031 (Ekins '031), and Chen et al., U.S. Patent 4,385,126 ('126).

3. I first met Professor Ekins at a meeting arranged in our office about 6 years ago. We were already aware of his work at that time, but were quite sceptical as to whether it would be applicable to the development of sensitive assays, since the notion of reducing the amount of binding agent in relation to the analyte to detect the analyte runs counter to the accepted concept that large amounts of binding agent are required to achieve high levels of analyte binding to gain maximum sensitivity. As a result of that meeting and subsequent discussions, we came to understand better the implications of the technology described in the above application (and Professor Ekins' other related patent applications) and realized in particular that it can allow

highly sensitive assays to be carried out using very small amounts of binding agent and involving incubation times at least as short as those of conventional assays.

4. It was our assessment that Professor Ekins' pioneering work had opened up an entire new approach in this field, allowing multi-analyte, miniaturized assays to be developed, which could be the basis for a new generation of assays, and that my company would have an excellent commercial opportunity if it developed them. We therefore negotiated an exclusive license under all of Professor Ekins' patents and patent applications relating to the ambient analyte technology. After having invested many tens of millions of dollars, we now have operational fully automated instrumentation and high volume manufacturing equipment. Commercial launch of the first system is planned for 1998.

5. Before the present invention was made, those of ordinary skill in the art, based on their experiences with competitive assays and the more widely practised non-competitive assays then in use, would not have been inclined to use binding agent concentrations of less than 0.1/K (i.e., amounts of less than 0.1 V/K) for the reason that this would be expected to lead to a substantial loss of sensitivity and yield assays requiring even longer incubation times than required in conventional assay methods. Practically, all recently introduced new systems, including one from our company, use microbeads as solid phase in order to increase the concentration of binder to 10 or even 100/K. I therefore believe that, notwithstanding the disclosure in Ekins '031, a person of ordinary skill in the art would find it surprising that the assay of the above application provides sensitivity enhancement (with shorter incubation times) by immobilizing small amounts of binding agent (less than 0.1 V/K moles) at high density in microspots. I believe it would not have been at all obvious to the person of ordinary skill in the art to do this to improve signal-to-noise ratios. As mentioned above, this runs against the conventional practice in the field of using large amounts of binding agent to obtain optimal sensitivity. Furthermore, I believe it is surprising that the sensitivity of an assay system increases as the size of the spot containing binding agent is reduced.

   In Example 2 of Ekins '031 the use of antibody having affinity constant of 2×10^{10} $1mol^{-1}$ (in an amount having a binding capacity of 10 fmoles) with samples of volume 0.2, 0.4 and 0.8 mls, represents the use of 1.0 V/K, 0.5 V/K and 0.25 V/K moles of binding agent. These amounts are all in considerable excess of the more stringent 0.1 V/K moles requirement of the above application.

   Even in light of Ekins '031, I do not believe that a person of ordinary skill in the art would have further reduced the amount of binding agent to the levels required in the above application, especially given the prejudice in the art against doing this and in the absence of any further suggestion or teaching that such a modification would produce any beneficial result.

6. Chen et al. '126 does not make up for the deficiency of the disclosure of Ekins '031, as Chen et al. '126 does not contain any disclosure concerning immobilizing less than 0.1 V/K moles of binding agent as a microspot, nor the advantages produced by this feature of the assay of the above application. I note in this regard that Chen et al, '126 fails to disclose or suggest that the use of such a small amount of binding agent

could provide assays of greater sensitivity, and having incubation times at least as short as assays of conventional design which require large amounts of binding agent, as noted above.

7. In any case, I do not believe that a person of ordinary skill in the art would have been motivated to apply the combined disclosures of Ekins '031 and Chen et al. '126 in such a way as to arrive at the assay of the above application.

Chen et al. provide essentially two reasons for utilizing dual labelling. First, antibody is labelled for the purpose of quality control, to ensure that the correct amount of antibody has been attached to the assay reagent and that the resulting reagent has not been damaged in transit (see col. 2, line 62 through col. 3, line 7 of Chen et al. '126). This procedure is described in Example 1 of Chen et al. '126. If the signal is found to be abnormally high or abnormally low, the data are disregarded (see col. 6, lines 29 and 30 of Chen et al. '126).

The other purpose for dual labelling disclosed by Chen et al. '126 is so that the signal omitted by the labelled antibody may be quantitatively detected independently of the detection of the labelled ligand bound to it. In this way, according to the disclosure of Chen et al. '126, the immunoassay may be made "self-calibrating" (see col. 3, lines 3-7 of Chen et al. '126). The reference to self-calibrating, however, is to the calibration of the fluorometer and not the calibration of the immunoassay by comparison with standard samples. In practice, a fluorescent tag on the antibody and a fluorescent tag on the back-titration agent are detected quantitatively while they are bound to each other, using the same fluorometer, and the quantity of ligand present in an unknown sample is determined as a function of the ratio of the quantitative measurements of the two tags. Any instrumental error or defect affecting the measurement of the strength of the fluorescent signal, particularly affecting the gain of the surface-to-fluorometer interface will affect both signals equally, according to Chen et al. and will leave the ratio unaffected (see col. 5, lines 13-24 of Chen et al. '126). This aspect of the procedure is described in Example 2 of Chen et al., with reference to the tables at columns 7 and 8, where two different types of background surface are compared, namely, clear glue and black glue, and measurements are given to show that the determination of the ratio of tag measurements can compensate for variable gain in the interface between the sample and the fluorometer (see col. 6, line 66 through col. 7, line 2 of Chen et al. '126).

In my view, the reasons given by Chen et al. '126 for using dual labels have essentially no relevance to the practice of the assay of the above application. As for the first reason, i.e., ensuring affixation of the correct amount of binding agent to a test surface during manufacture, the assay of the above application is independent of the amount of binding agent used (see page 11, lines 7-23 of the above application). Thus, ensuring affixation of any specific amount of binding agent is unnecessary. In regard to the second reason, i.e., rendering the assay procedure self-calibrating with reference to instrument measurement, although this feature may be applied to any conventional assay format it provides no particular advantage for the assay of the above application. In order to properly work Chen's dual label assay requires that the function of the binder, namely, the antigen-antibody interaction, nucleic acid hybridization, etc, is strictly related to the intensity of the label on the binder. It is

fairly easy to understand that this will not be the case, especially considering the stability requirements of such an assay. Therefore, this dual label approach would never be used in an assay embodying the invention of the above application.

8. In summary, the disclosure of Ekins '031 and Chen et al. '126, considered individually or together, make no reference to assay sensitivity and, therefore, provide no teaching in the direction of the present invention to the person of ordinary skill in the art.

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true and further that these statements were made with the knowledge that wilful false statements and the like so made are punishable by fine or imprisonment, or both, under §1001, Title 18 of the United States Code, and that such wilful false statements may jeopardize the validity of this application or any patent issued thereon.

Date 6 NOV 97

Johann Berger

JOHANN BERGER

Die/Das vorstehende(n) Unterschrift(en)/Handzeichen wurde von
Herrn Dr. Berger Johann
wohnhaft in Seehaupt.
(Ort u. Straße)
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